

Characterization - it matters, but what and how?



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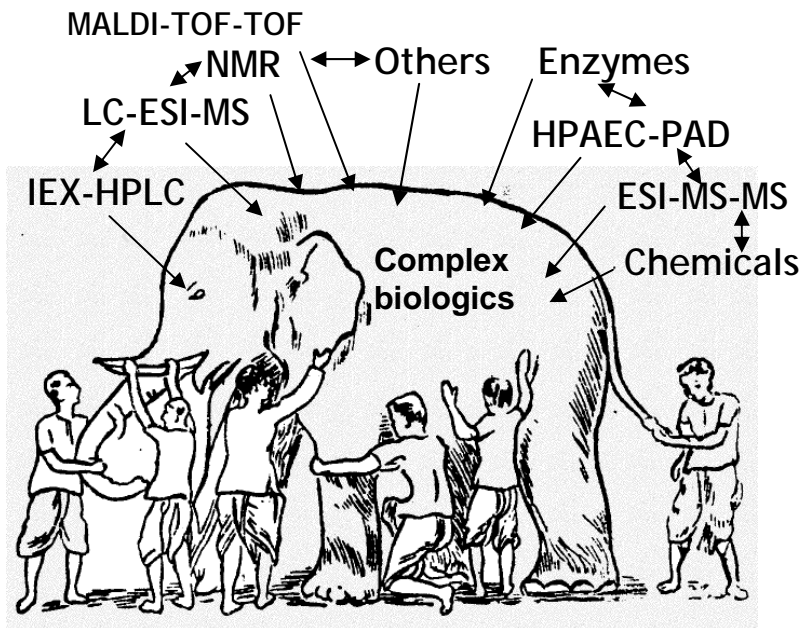


Outline

- Framework
 - Simple molecules to complex biologics
- Glycans do matter
 - Why and how?
- Characterization
 - Issues and challenges
 - Newer approaches
- Equivalence for complex biologics
- Summary

Complex biologics

The Challenge



American poet John Godfrey Saxe (1816-1887) based the following poem on a fable which was told in India many years ago. It is an example of how limited sensory perceptions can lead to misinterpretations

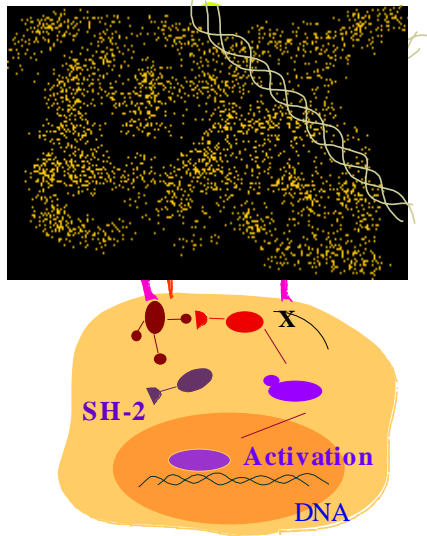
Moral:

*So oft in theologic wars,
The disputants, I ween,
Rail on in utter ignorance
Of what each other
mean,
And prate about an Elephant
Not one of them has
seen!*

<http://courses.cs.vt.edu/~cs1104/Introduction/6.blind.men.html>

Complexity: Old to New Biology

Reductionism



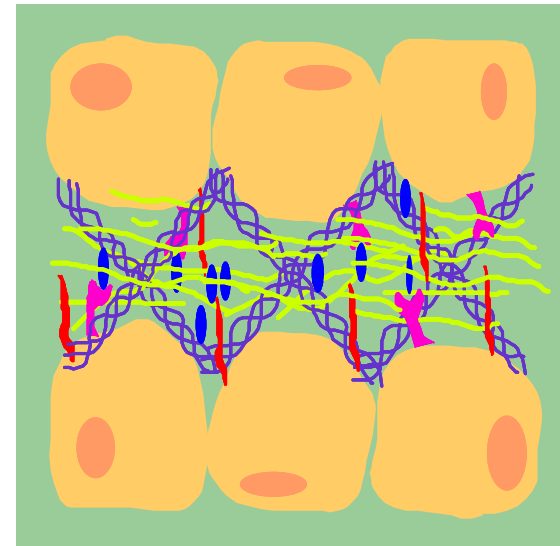
<http://web.mit.edu/tox/sasisekharan/>

DNA-RNA-Proteins

Technology:

**DNA and Protein sequencing
& Recombinant DNA**

Integrated Systems View



<http://web.mit.edu/tox/sasisekharan/>

Genomics, Proteomics

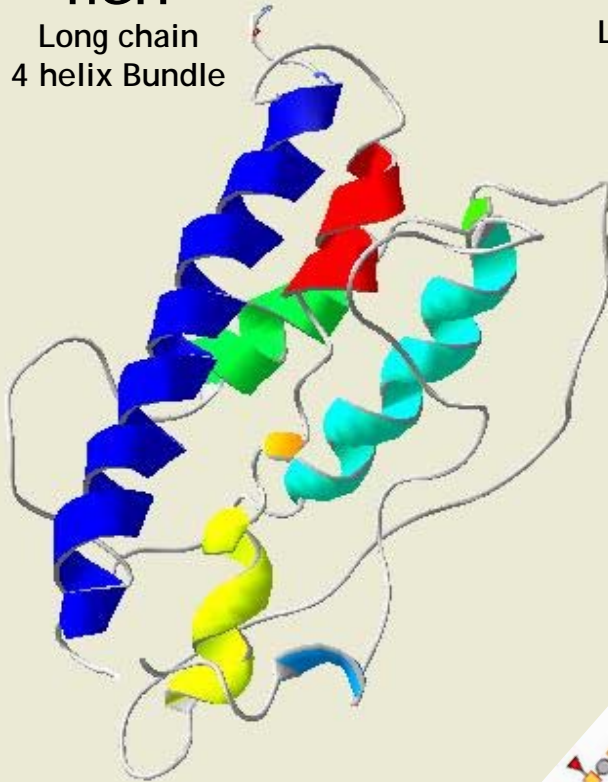
Glycomics

**Technology: HT robotics
Whole Organism Genetics
Informatics**

Spectrum of Complexity

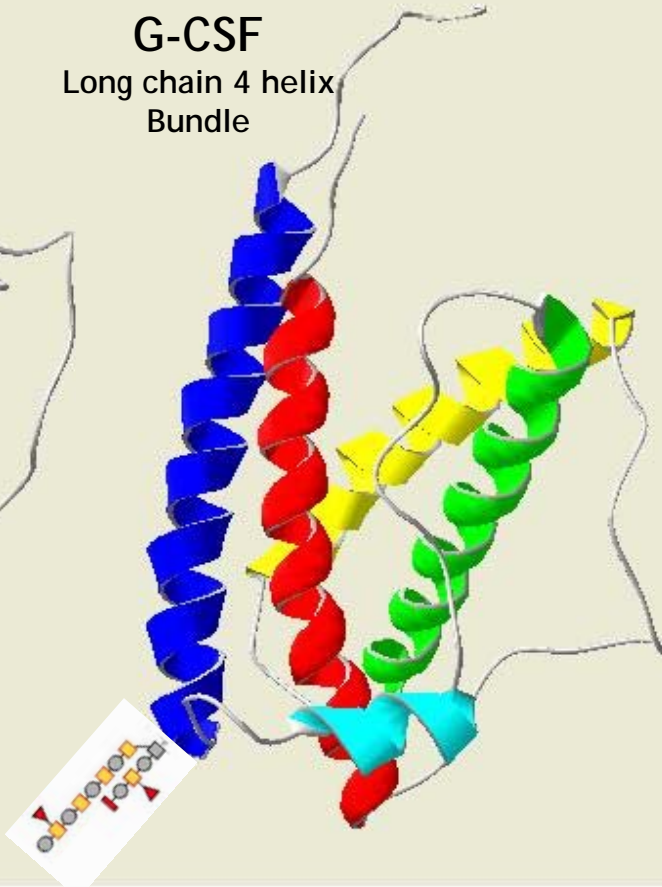
Structures from PDB

hGH
Long chain
4 helix Bundle



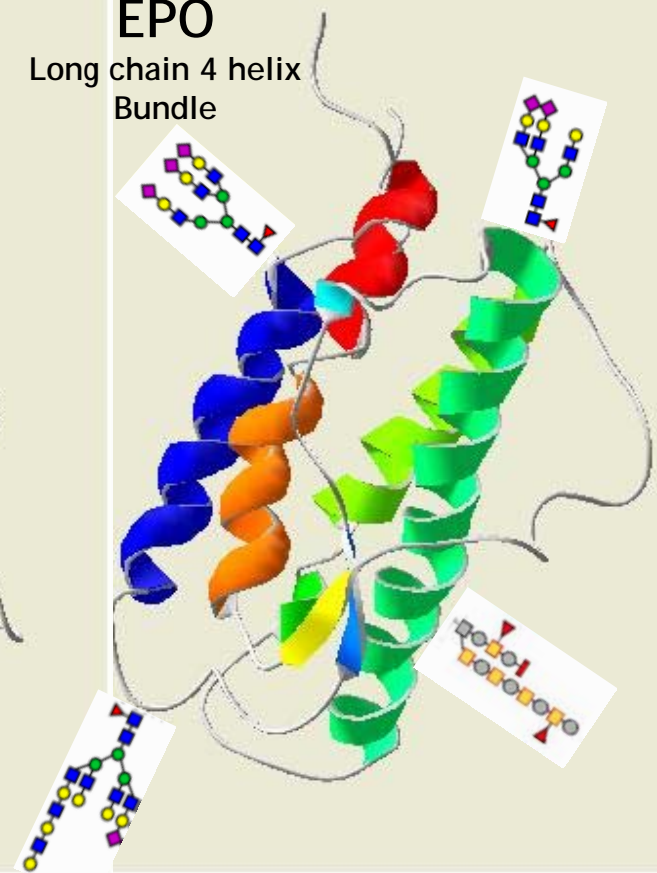
No glycosylation

G-CSF
Long chain 4 helix
Bundle



1 O-glycosylation

EPO
Long chain 4 helix
Bundle



1 O-glycosylation
3 N-glycosylation



Glycosylation

case in point for follow-on biologics

Not new - actually has been more a problem esp. for protein based therapeutics -

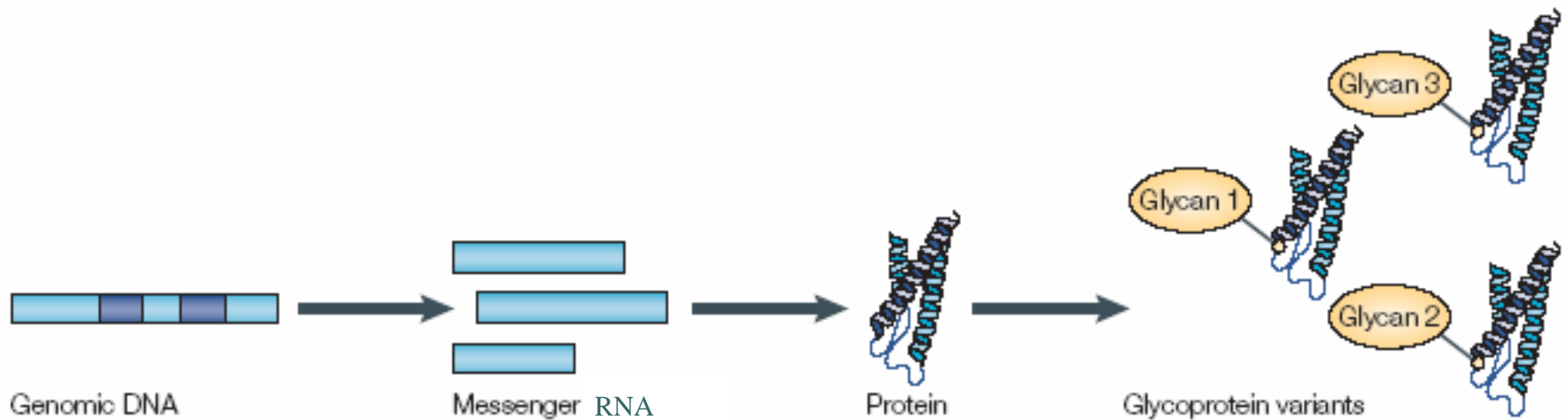
- “Glycosylation is a hassle to deal with”
- “Quality of the protein can be influenced by the degree and differences in the oligosaccharide structure”
- Glycans are ‘a gimish’

Some Important Lessons

- Cannot view glycans as “impurities or just chemicals”
- Glycosylation affects folding and hence - immunogenicity [INF β , GM-CSF]
- Glycosylation affects pK/pD parameters [EPO, G-CSF]
- Up to 35% wt of proteins can be glycans!!
- Heterogeneous, polydisperse, high information content due to diversity in the chemical structure - hence ensemble of structures

One gene... one protein

... many glycoproteins



Functional diversity

Nature Review Drug Discovery. 2004(10):863-73

Glycans and Diseases

Renaissance for glycans

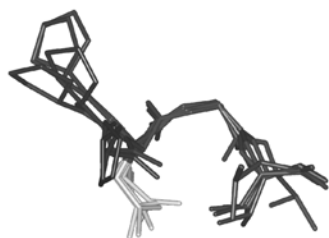
Hereditary Multiple
Exostoses



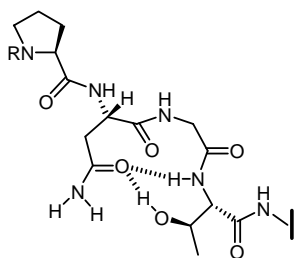
Simpson-Golabi-Behmel
Syndrome

Glycosylation Effects on Protein Conformation

Unglycosylated

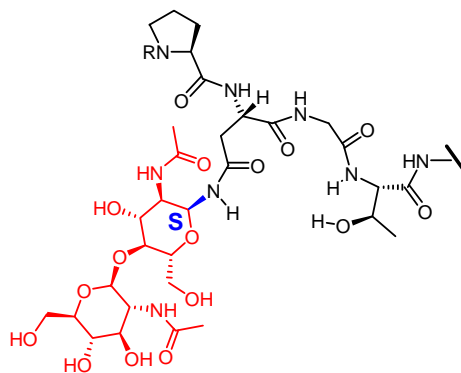
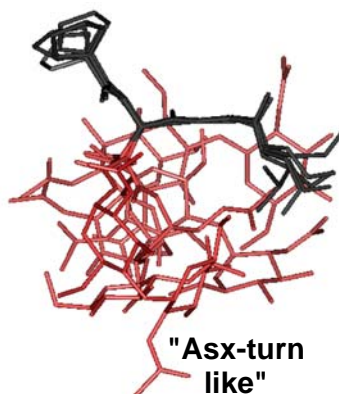


Asx-turn



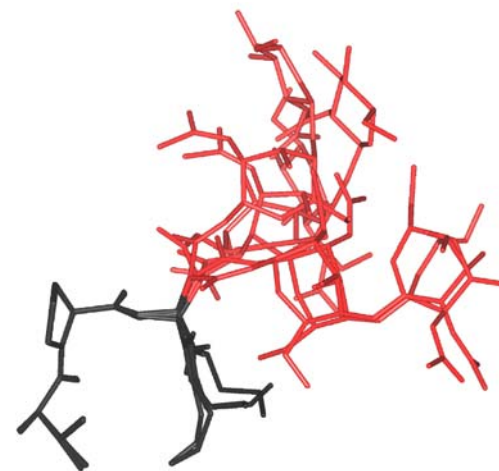
1

α -linked glycosylation

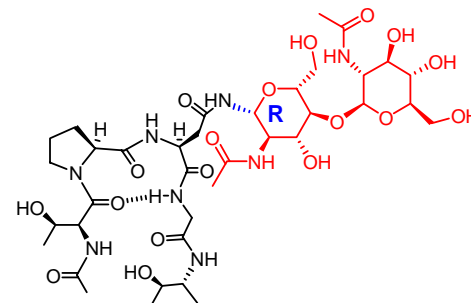


1- α

Natural β -linked
glycosylation
Type I- β turn



Type
I- β turn

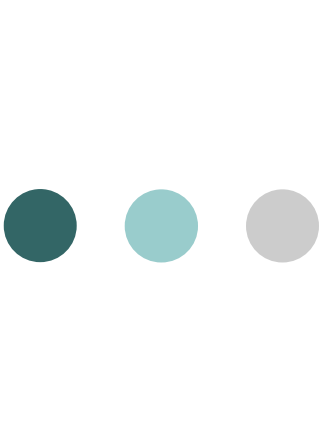


1- β

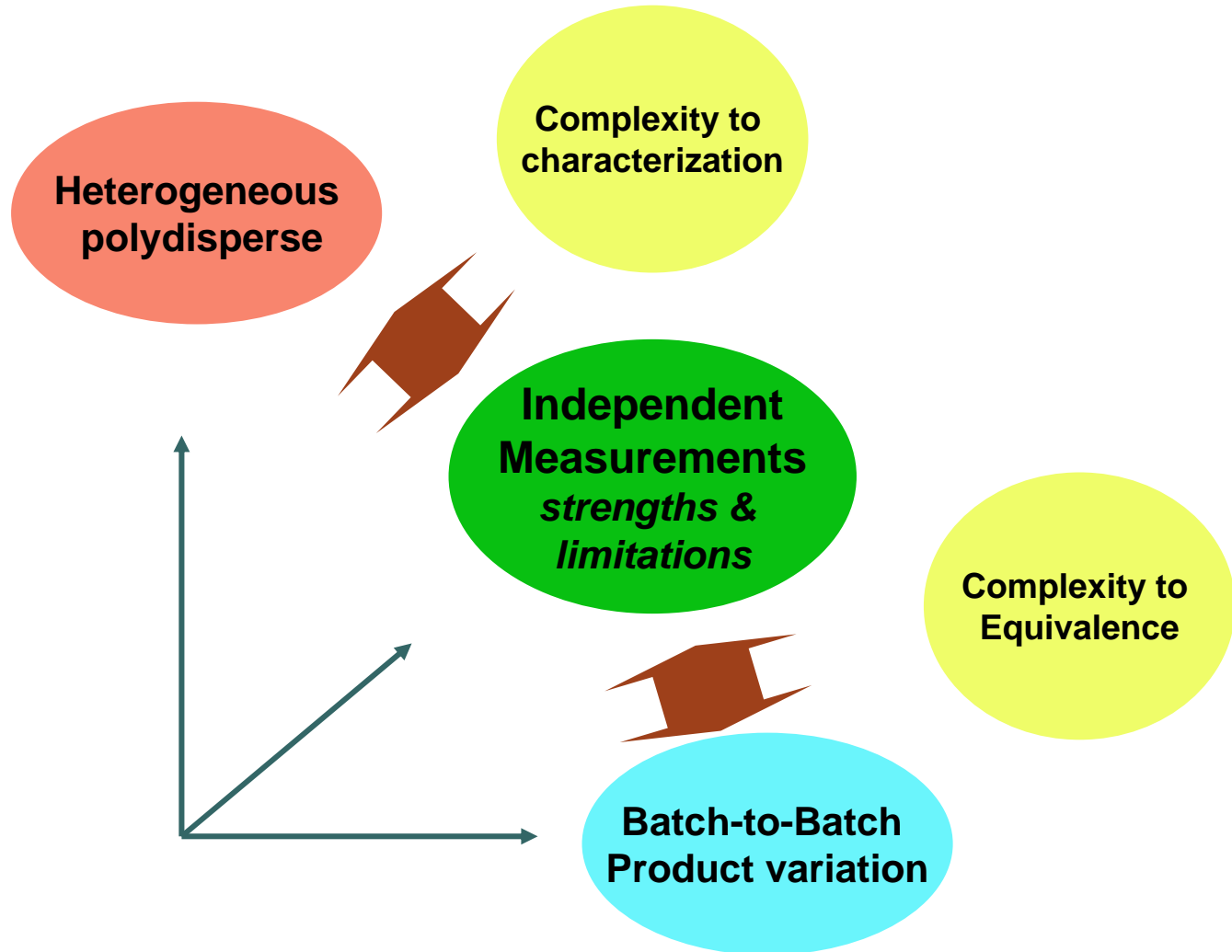
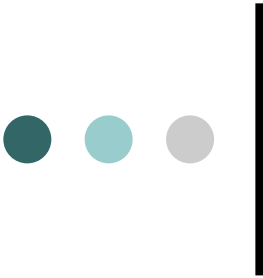


Glycans are critical to clinical profile

- Glycans modulate
 - Protein folding and stability
 - Binding activity to receptors and other biomolecules, influencing efficacy and safety
 - Immunogenicity through folding
 - Pharmacokinetics
 - Tissue distribution
- Contributions of glycans to clinical profile are similar in importance to amino acid sequence and protein structure
- Thus, thorough characterization of glycans needs to be required by FDA for approval of follow-on biologics



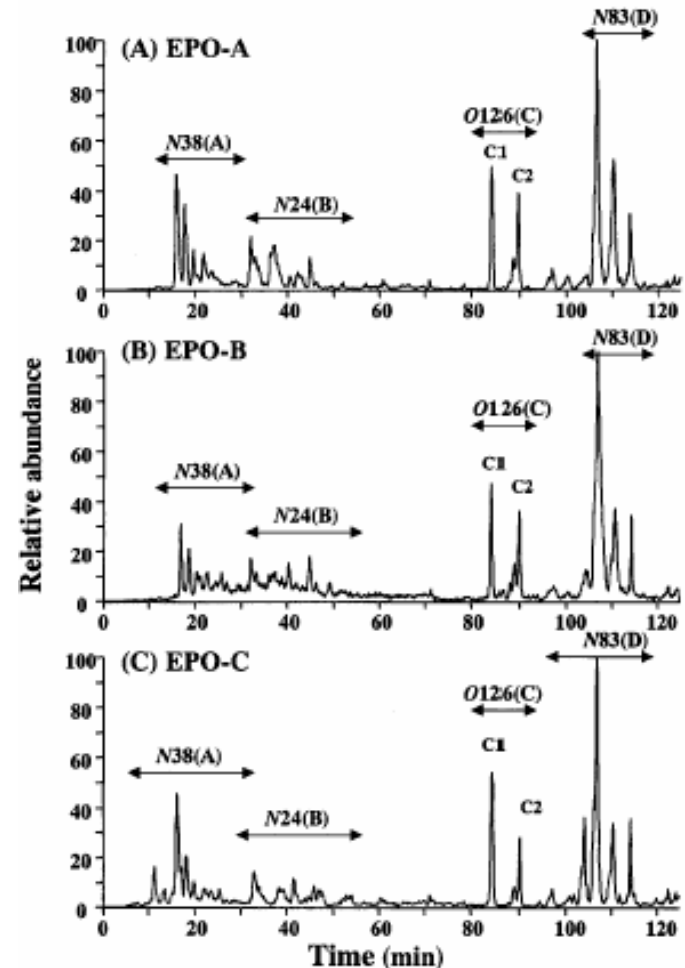
Characterization & Equivalence



Glycan Technology

Capabilities & Limitations

- Glycan released from proteins and analyzed
 - Chemical methods (non specific cleavage)
 - Enzymatic methods (PNGase-F etc)
 - Non-specific cleavage or cannot access some sites
- Compositional analysis
 - Monosaccharide analysis
 - HPLC, PAD detection
 - Mass Spectrometry
 - Sialic acid content analysis
 - Separation and resolution can be challenging due to overlapping peaks

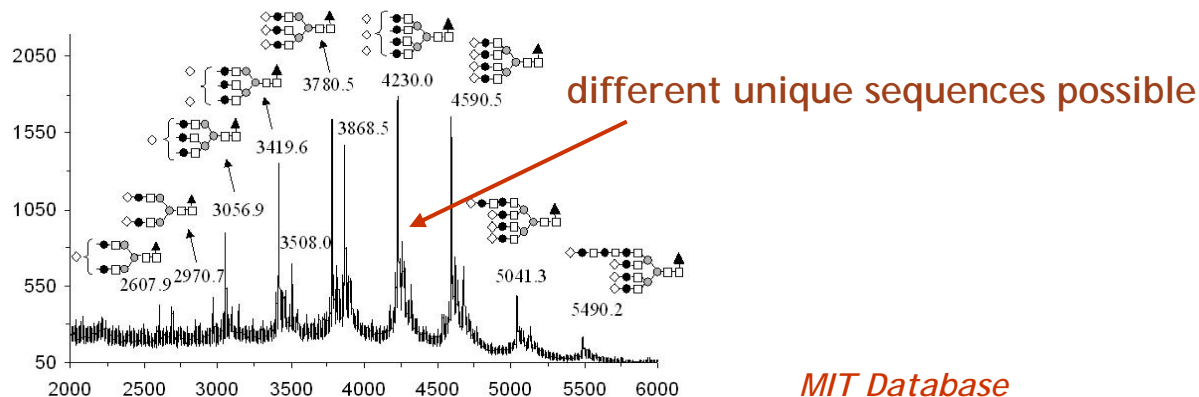


Biologicals. 2002 Sep;30(3):235-44

Glycan Technology

Capabilities & Limitations

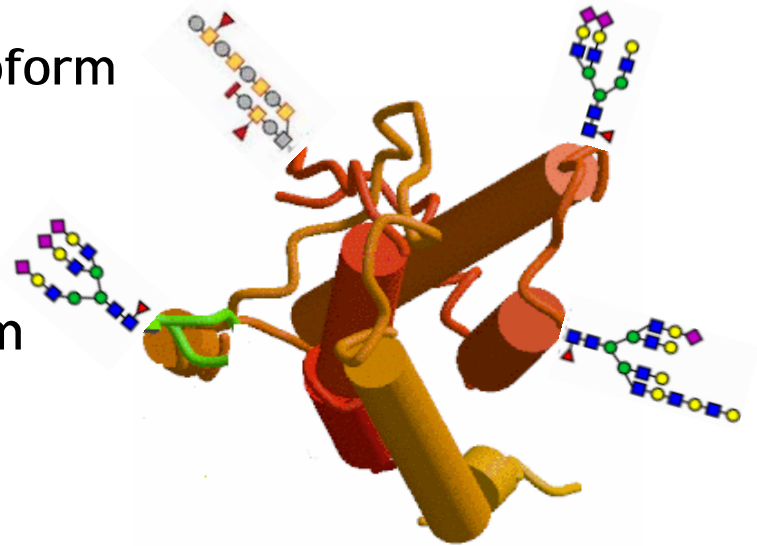
- Distribution analysis
 - Comparison of traces to determine reproducibility in manufacture
 - Isomeric structures (same composition, different linkage)
- Molecular weight analysis and sequencing of major components
 - MALDI MS, MW distributions
 - MALDI-MS, ESI-MS, PSD (MS_n)
 - Low abundance species often not accounted for including minor modifications



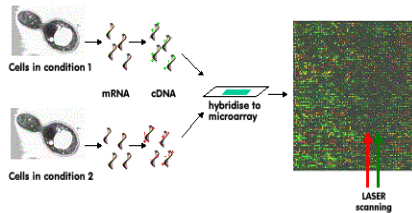
Glycan Characterization

Needs or requirements

- Requirements for equivalence
 - Low abundance species [to meet FDA requirements]
 - Associating glycoforms with their sites of glycosylation
 - Accurately quantifying each glycoform
 - Analyzing subtle modifications
 - Fucosylation
 - Sulfation
- Determining sensitivity of glycoform structures
 - Process conditions
 - Protein properties

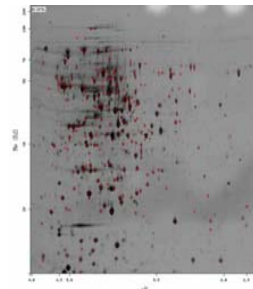
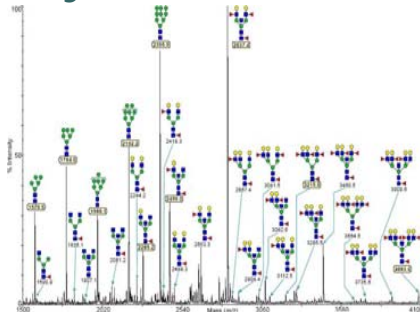


Glycomics: *a systems approach to study glycans*

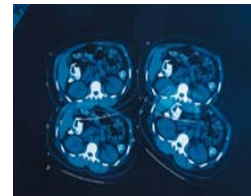


Genomics

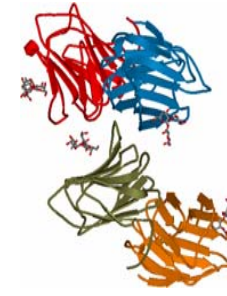
Glycomics



Proteomics



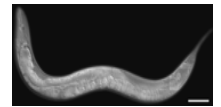
Cells



Molecule



Organisms



Tissues/Organs

TOOLS

TARGETS

Data Integration with Informatics platform

Glycomic Database

Relational databases

Genomic

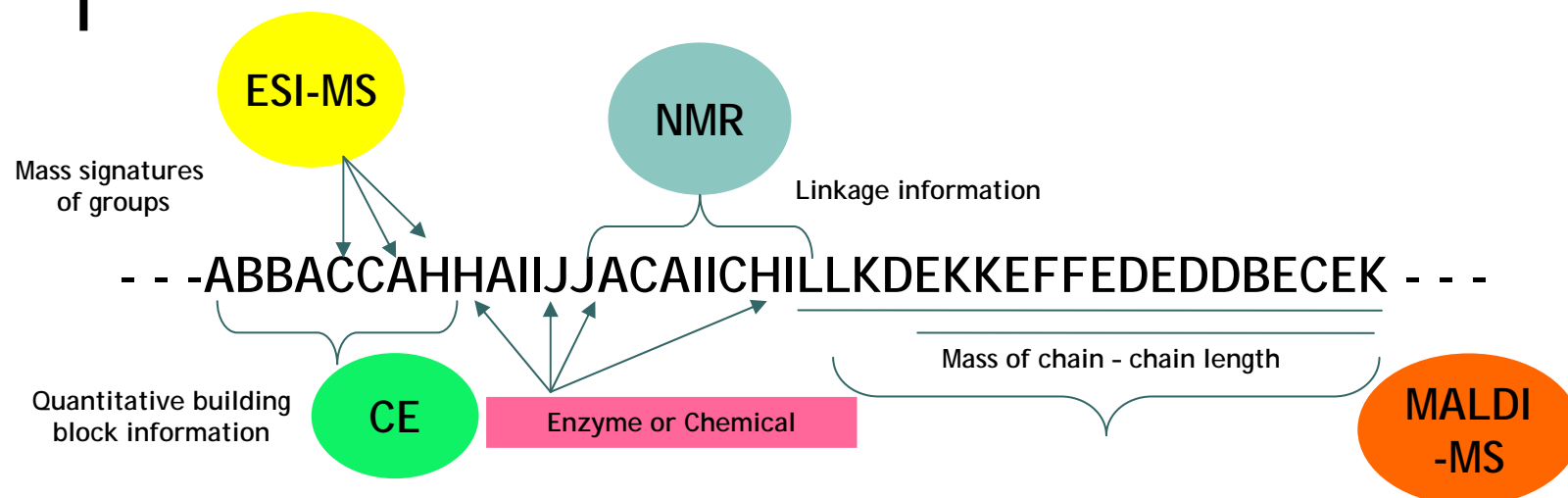
Proteomic

Other

Molecule Pages

Home > Molecule Pages > Glycan Binding Proteins > Gallectins > Galactin-3 (human)	
General	Resources
<p>CFG ID: chr_hum_galact_0006</p> <p>CRP Name: Galactin-3</p> <p>Category: Galactin Family</p> <p>Other names: MAC-2 antigen, CDP-35, m1-34, L-29, NL-31, epsilon BP, IgG binding protein</p> <p>Species: Human</p> <p>Summary: Galactin-3 contains a carboxyl-terminal lectin domain and an amino-terminal non-lectin part consisting primarily of short random repeats. It is widely distributed in tissues and found in epithelial cells, fibroblasts, dendritic cells, and inflammatory cells. In many cell types studied, galactin-3 is present diffusely in the cytoplasm, but is also localized to the nucleus and subcellular structures, such as mitochondria, phagosomes and endosomes, under specific conditions. It is secreted by various cell types, including monocytes, macrophages, and epithelial cells, and the extracellular protein can bind to a large number of different glycosylated proteins on the cell surfaces and extracellular matrices. Galactin-3 can form dimers through intermolecular interactions that involve the N-terminal domain and can function as a dimer. It has the potential to cross-link cell surface glycoproteins of various cells, causing cell activation (such as mediator release and superoxide production). It is also suited for mediating cell-cell and cell-extracellular matrix adhesion (including homophilic cell aggregation) by serving as a bridge to bind cells together or cells to extracellular matrix proteins. Moreover, it can induce migration of a number of different cell types, including monocytes, macrophages, and endothelial cells, possibly through binding to and activating a G-protein-coupled receptor. Galactin-3 is present on the cell surface. Cell surface galactin-3 on T cells can form multivalent complexes with its glycans on TCR and thereby restricts the lateral mobility of TCR complexes and suppress TCR-mediated signal transduction. Galactin-3 is also abundantly present inside the cells and has been shown to play important roles in some biological responses through its intracellular actions. It has been identified as a regulator of the cell cycle, apoptosis, and phagocytosis. The mechanisms underlying these functions have not been elucidated, but they probably involve the</p>	

Integration of Methods



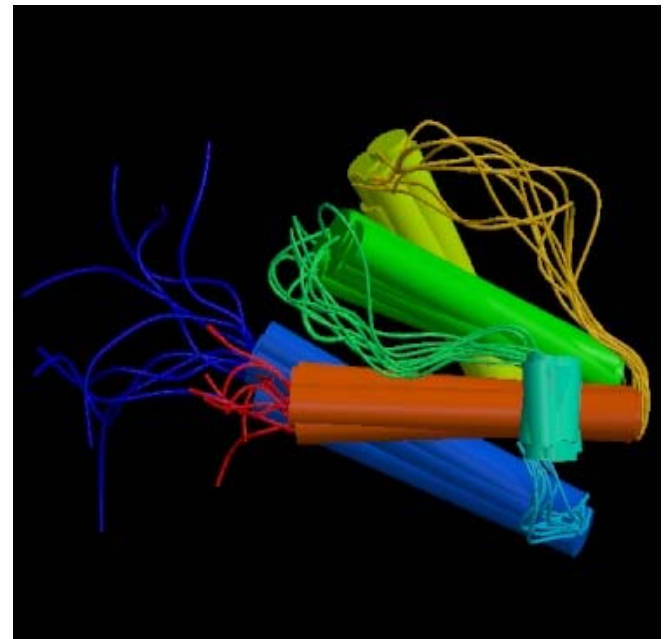
IG	2X	6X	3X	NX	CODE	UNIT	MASS (ΔU)
0	0	0	0	0	0	I-HNAc	379.33
0	0	0	0	1	1	I-HNS	417.35
0	0	0	1	0	2	I-HNAc,3S	459.39
0	0	0	1	1	3	I-HNS,3S	497.41
0	0	1	0	0	4	I-HNAc,6S	459.39
0	0	1	0	1	5	I-HNS,6S	497.41
0	0	1	1	0	6	I-HNAc,3S,6S	539.45
0	0	1	1	1	7	I-HNS,3S,6S	577.47
0	1	0	0	0	8	I2S-HNAc	459.39
0	1	0	0	1	9	I2S-HNS	497.41
0	1	0	1	0	A	I2S-HNAc,3S	539.45
0	1	0	1	1	B	I2S-HNS,3S	577.47
0	1	1	0	0	C	I2S-HNAc,6S	539.45
0	1	1	0	1	D	I2S-HNS,6S	577.47
0	1	1	1	0	E	I2S-HNAc,3S,6S	619.51
0	1	1	1	1	F	I2S-HNS,3S,6S	657.53



Science, 1999
PNAS, 2000, 2001, 2002
Nature Medicine 2001
Nature Reviews Drug
Discovery 2204

Protein Characterization

- Currently, many different analytical tools allow thorough characterization of protein structural properties
 - Primary, secondary, tertiary, quaternary structures [including subtle conformational features]
- State-of-the-art analytical techniques allow investigation of protein of physicochemical and biochemical properties
 - This includes chemical modifications or alterations etc.
- Several orthogonal techniques are available and can be readily used to address complexity of both structural and biochemical properties of proteins

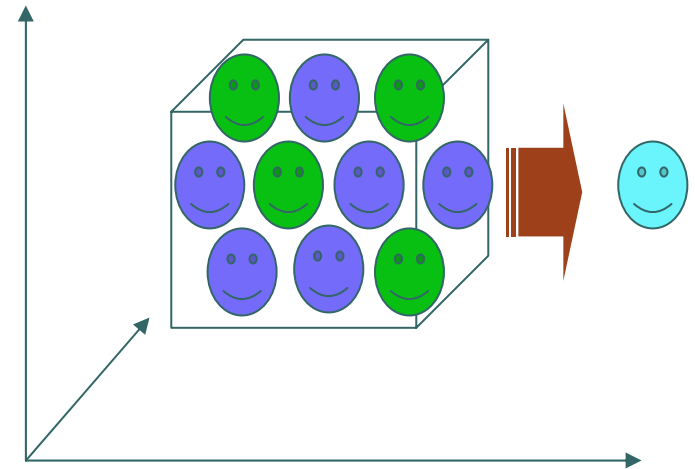


Source: Protein Databank

Equivalence Window

for complex biologics

- By definition - biologics are heterogeneous mixtures and vary batch to batch
- We cannot 'average' data
- Quantitative relationship between different batches can be determined.
- Quantitative windows that can define equivalence can be obtained based on these metrics.
- Quantitative window of equivalence will help determine if two batches of complex biologics are equivalent.





Summary

- Glycans play critical role in biology and chemistry of proteins
- Thorough characterization of sugars, commensurate with that of proteins, is critical for assuring sameness
- Technology makes possible for thorough characterization
- Paradigm for analyzing glycans can be extended to whole issue of identifying equivalence - new way of thinking about a complex problem